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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/390,634	09/07/99	PRICE	P 0942.4190002

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EXAMINER	
KERR, J	
ART UNIT	**PAPER NUMBER**
1633	7

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Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 09/390,634	Applicant(s) Price et al.
	Examiner Janet M. Kerr	Group Art Unit 1633

☒ Responsive to communication(s) filed on *Aug 9, 2000* .

☒ This action is **FINAL**.

☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *3* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *89-126* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *89-126* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____ .

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____ .

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☐ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

Response to Amendment

Applicants' amendment, filed 8/9/00, has been entered.

Claims 89-126 remain pending.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 89-126 remain rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for methods of culturing murine embryonic stem cell lines in a serum-free medium composition comprising the specific nutrients and ranges of nutrients recited in the preferred embodiments of Tables 1-3, as set forth in Examples 1-5, and compositions comprising said embryonic stem cells and said medium, does not reasonably provide enablement for methods of culturing any embryonic stem cell, in the absence of a feeder layer, and with any serum-free culture medium, compositions comprising any embryonic cell with any serum-free medium formulation, methods of differentiating embryonic stem cells under serum-free conditions, methods of obtaining embryonic stem cells from blastocysts using serum-free culture conditions, or methods of producing recombinant proteins using embryonic stem cells under serum-free conditions for the reasons of record and the reasons below. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Claims 89, 91, and 105-107 are directed to methods of expanding embryonic stem cells in serum-free culture. Claims 108-116 are directed to methods for controlling or preventing the differentiation of embryonic stem cells in serum-free culture. Claims 117-121 are directed to methods of differentiating embryonic stem cells into a particular cell type. Claims 122 and 123 are directed to methods of obtaining embryonic stem cells in serum-free culture. Claims 124-126 are

directed to methods of producing recombinant proteins in embryonic stem cells in serum-free culture.

Claims 91-97 are directed to compositions comprising embryonic stem cells in a serum-free medium.

Claims 98-104 are directed to products of manufacture comprising embryonic stem cells and serum-free medium and a eukaryotic cell culture medium supplement.

While the specification is enabling for methods of culturing the embryonic stem cell lines, D3 ES cell line, the two mouse strain 129 ES cell lines, E14 and R1, and a non-129 ES cell line, TT2, under defined culture conditions as set forth in Examples 1 through 5, the specification is not enabling for culturing any embryonic stem cell in the absence of a feeder layer utilizing the broadly claimed medium supplements, nor is the specification enabling for methods of differentiating embryonic stem cells, methods of isolating embryonic stem cells from blastocysts, or methods of producing recombinant proteins as claimed. The specification discloses culturing the above-mentioned embryonic stem cell lines in a particular serum-free formulation (see Examples 1 through 5, and Tables 1 and 3). Cells cultured under these specific conditions are capable of expansion, *in vitro*, and display normal phenotypic and morphologic characteristics. The specification also provides an example of germ-line competence with R1 ES cells grown under these specific conditions (see, e.g., Example 8).

However, with regard to the broadly claimed embryonic stem cells, at the time of filing, the state of the art is such that generation of ES cells, i.e., cells which retain their totipotential capacity and are able to generate cells of all lineages, including germline, after being introduced into host a blastocyst, is neither routine nor predictable in species other than mice. Keller (Current Opinion in Cell Biology, 1995) teaches that ES cells are totipotent lines derived from the inner cell mass of developing blastocysts. When maintained on embryonic fibroblasts in culture, ES cells retain their totipotential capacity and are able to generate cells of all lineages, including germline, after being introduced into a host blastocyst (see page 862, left column, first paragraph). However, at the time of filing, the state of the art is such that generation of ES cells, i.e., cells

which retain their totipotential capacity and are able to generate cells of all lineages, including germline, after being introduced into host a blastocyst, is neither routine nor predictable in species other than mice. Bradley (Biotechnology, 1992) teaches that while a number of reports have been made claiming isolation of ES cells from farm animals, the description of these cell lines is yet to be supported by the demonstration that they can proliferate and differentiate in an embryo, *in vivo*, contributing to somatic tissues or germ cells (see page 53, right column, last paragraph bridging page 54). Moreover, Seamark (Reproductive Fertility and Development, 6:653-657, 1994) teaches that totipotency for ES cell technology in many livestock species has not been demonstrated (see, e.g., Abstract). Similarly, Matsui *et al.* (Cell, 1992) disclose that while it is well established that pluripotential stem cells, i.e., those originally termed ES cells, can be derived from the epiblast of blastocysts in culture, it is crucial to determine whether blastocyst-derived stem cells differ in their full range of developmental potencies and properties, such as genomic imprinting (see page 845, right column, 2nd paragraph, under "Reprogramming...", and page 846, left column, second full paragraph). In view of the lack of guidance in the specification with regard to the process of obtaining embryonic stem cells with the requisite "embryonic stem cell properties", the use of the cells in the claimed cell culture method and the claimed products of manufacture is not enabled.

The claims are also non-enabled as they broadly recite supplement ingredients to be used in the claimed culture methods and the claimed products of manufacture, i.e., supplement ingredients which are capable of supporting growth of embryonic stem cells. While the specification discloses a particle medium formulation containing the supplement ingredients at specific concentrations, and provides evidence that such a medium formulation supports embryonic stem cell growth/differentiation, the specification does not disclose or provide evidence that the broadly claimed supplement ingredients at any concentration range support the growth/differentiation of embryonic stem cells. The specification fails to provide an enabling disclosure for how to make and use any and all combinations of media supplements, media formulations, and media useful for the claimed methods as the specification does not provide

adequate guidance for the selection of appropriate serum-free medium supplements that would support the expansion/differentiation of embryonic stem cells as required by the claims. While the specification provides an explicit teaching regarding the ingredients that could be used to make a medium appropriate to practice the claimed invention, the specification does not provide guidance as to which ingredients can be excluded from the formulation, and whether the concentrations of the remaining ingredients need optimization as a result of the exclusion of particular ingredients.

Moreover, the state of the art at the time of filing indicates the difficulties of culturing embryonic stem cells. For example, Baribault *et al.* (Mol. Biol. Med., 1989) disclose that culture conditions and passage number may influence the ability of ES cells to give rise to germ-line chimeras. Different conditions might induce changes in karyotype, or culture of ES cells for many passages may select for the cells with a higher growth rate and teratocarcinoma-like phenotype. Baribault *et al.* also teach that when embryonal carcinoma cells are re-injected into mouse blastocysts, they infrequently produce germ-line chimeras but often induce tumors. A further consideration of culturing ES cells under conditions for maintaining an undifferentiated state is the stability of the stem cell phenotype, which should be monitored using cell surface markers which identify the embryonic phenotype, as well as regular karyotyping (see page 485, left column, first paragraph).

Taken together, the state of the art at the time of filing establishes the unpredictability of obtaining embryonic stem cells and maintaining such cells in culture such that the cells retain the requisite "stem cell characteristics", i.e., the ability to proliferate and differentiate in an embryo, *in vivo*, contributing to somatic tissues or germ cells. Inasmuch as the specification only discloses one particular medium formulation which supports the growth of specific embryonic stem cell lines, D3 ES cell line, the two mouse strain 129 ES cell lines, E14 and R1, and a non-129 ES cell line, TT2, such that said cells maintain a stable phenotype and retain their totipotential capacity such that they are able to generate cells of all lineages, including germline, after being introduced into a host blastocyst, one of skill in the art make or use the invention as claimed without undue experimentation.

Claims 117-121, directed to differentiating the cells in serum-free culture are also not enabled as the specification clearly indicates, on page 44, that when the cells are plated on electrostatically charged plastic and allowed to attach, the embryoid bodies would not attach without the addition of 1% FBS to supply undefined attachment factors. Once attached, the differentiated cells that grew out of the embryoid bodies were quite different than those seen in FBS-supplemented medium. While the specification indicates that cells can be induced to differentiate to at least cardiac cells, it is apparent from the specification that serum is required to effect differentiation. Thus, in view of the teachings in the specification, and the absence of guidance as to serum-free ingredients which can be used to replace the 1% FBS, one of skill in the art could not practice the invention as claimed without undue experimentation.

Applicant's arguments filed 8/9/00 have been fully considered but they are not persuasive. It is argued that the examiner has not established a *prima facie* case of non-enablement, as the examiner has not provided objective evidence or sound scientific reasons why the claimed invention is not enabled. Specifically, on page 11 of applicants' Response, it is argued that applicants define the term "embryonic stem cell" and "pluripotent embryonic stem cell" to refer to a cell which can give rise to many differentiated cell types in an embryo or an adult, including the germ cells. Applicants assert that the claimed methods, compositions, and product of manufacture relate to in vitro expansion of embryonic stem cells, not to the in vivo effect of embryonic stem cells that have been inserted into a host animal blastocyst. It is argued that the examiner has misinterpreted the claims to include an *in vivo* limitation. This is not persuasive. The claimed invention requires embryonic stem cells. As recognized in the art at the time of filing, the definition of embryonic stem cells are cells which retain their totipotential capacity and are able to generate cells of all lineages, including germline, after being introduced into host a blastocyst. Obtaining embryonic stem cells from species other than mice, is known in the art to be neither routine nor predictable as taught by Bradley, Seamark, and Matsui *et al.*

It is asserted that the examiner has not provided any evidence or technical reasons why one of ordinary skill in the art would have doubted that embryonic stem cells could have been identified based on morphological characteristics, the ability to be maintained in culture in an undifferentiated state, or the ability to control the differentiation of the cells in culture (see page 12 of applicants' Response). Applicants have provided pre-filing date literature with examples of embryonic stem cell lines obtained from multiple strains of mice and from non-mouse species (see pages 13-15). These arguments are not persuasive. With regard to the identification of embryonic stem cells, as previously indicated, an embryonic stem cell is defined as cells which retain their totipotential capacity and are able to generate cells of all lineages, including germline, after being introduced into host a blastocyst. To date, only mouse embryonic stem cells have been routinely isolated and characterized to this extent.

With regard to the literature provided by applicants, it is noted that the Doetschman *et al.* Reference indicates that experiments were being carried out to determine if the hamster ES cells can colonize the germ line when introduced into hamster blastocysts (see page 227, left column, last paragraph). Graves *et al.* teach that the it remains to be tested whether these a well as other presumptive ES cells (hamster, pig, sheep, cow, and mink), are totipotent, i.e., if the cells can give rise to viable progeny when introduced into recipient blastocysts (see page 432, left column). Iannaccone *et al.* teach that "Strain differences in the frequency of establishing continuous ES cell lines in the mouse are well known, and although it is reasonable to suppose that this is also true in the rat, at this time we have no formal evidence that it is. These are important considerations since as yet our chimeras have not demonstrated germ line transmission" (see page 291, right column). The reference of Notarianni *et al.* is directed to pluripotential embryonic cell lines from pig, not embryonic stem cells. The reference of Strelchenko *et al.* is directed to pluripotential embryonic cell lines from bovine, not embryonic stem cells. With regard to the references directed to establishment of mouse embryonic stem cells, as indicated in the above rejection, establishment of mouse embryonic stem cells is known in the art. In view of the teachings of Bradley, Seamark, and Matsui *et al.*, and further taught in the references supplied by applicants,

isolation of true embryonic stem cells from species other than mouse is neither routine nor predictable.

It is argued that only routine experimentation would have been required to develop serum-free cell culture media that support the expansion of embryonic stem cells *in vitro*. Applicants assert that given the disclosure in the instant application, one of ordinary skill in the art would know the necessary starting points from which formula ingredients and concentrations may be optimized. Applicants rely on the teachings of Freshney, and Jayme *et al.*, and Ham *et al.* to support the argument that only routine experimentation would have been required to formulate appropriate medium having the claimed characteristics and to practice the claimed methods (see pages 15-20 of applicants' Response). These arguments are not persuasive. The specification discloses one particular medium formulation which when used in culturing mouse embryonic stem cells or mouse embryonic stem cell lines, wherein the embryonic stem cells are maintained on a feeder layer, the cells are capable of expansion *in vitro* in the absence of serum, and maintain the appropriate morphologic and phenotypic characteristics. Moreover, these cells were able to colonize the germ line when introduced into mouse blastocysts. However, the specification does not disclose that culture medium containing less than the ingredients and concentrations listed in Tables 1-3 would support mouse embryonic stem cells *in vitro*. As previously stated, Baribault *et al.* teach that culture conditions and passage number may influence the ability of ES cells to give rise to germ-line chimeras. Different conditions might induce changes in karyotype, or culture of ES cells for many passages may select for the cells with a higher growth rate and teratocarcinoma-like phenotype. Baribault *et al.* also teach that when embryonal carcinoma cells are re-injected into mouse blastocysts, they infrequently produce germ-line chimeras but often induce tumors. A further consideration of culturing ES cells under conditions for maintaining an undifferentiated state is the stability of the stem cell phenotype, which should be monitored using cell surface markers which identify the embryonic phenotype, as well as regular karyotyping (see page 485, left column, first paragraph). While one of ordinary skill in the art could readily manipulate medium formulations, given the unpredictability in the art of culturing embryonic stem

cells, it would require undue experimentation to formulate a medium composition which supports *in vitro* expansion of embryonic stem cells. The breadth of the claims is not commensurate in scope with the teachings in the specification.

With regard to claims 117-121, it is argued that no evidence or sound technical reasons have been provided as to why one of ordinary skill in the art would not have been able to practice the methods of claims 117-121 without undue experimentation (see pages 21-22 of applicants' Response). This is not persuasive. As indicated above, the specification clearly indicates that serum is necessary to practice the claimed invention. As the claims are directed to a serum-free culture environment, the cells could not differentiate as required, using the claim-designated methods.

No claims are allowed.

THIS ACTION IS MADE FINAL. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for response to this final action is set to expire THREE MONTHS from the date of this action. In the event a first response is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event will the statutory period for response expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Janet M. Kerr whose telephone number is (703) 305-4055. Should the

examiner be unavailable, inquiries should be directed to Deborah Clark, Supervisory Primary Examiner of Art Unit 1633, at (703) 305-4051. Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center number is (703) 305-7401. Any administrative or procedural questions should be directed to Kimberly Davis, Patent Analyst, at (703) 305-3015.

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